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# High-runner mice have reduced incentive salience for a sweet-taste reward when housed with wheel access

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ABSTRACT

To explore reward substitution in the context of voluntary exercise, female mice from four replicate high-runner (HR) lines (bred for wheel running) and four non-selected control (C) lines were given simultaneous access to wheels and palatable solutions as competing rewards (two doses of saccharin [0.1, 0.2% w/v]; two doses of common artificial sweetener blends containing saccharin [Sweet 'N Low®: 0.1, 0.2% w/v], aspartame [Equal®: 0.04, 0.08% w/v], or sucralose [Splenda®: 0.08, 0.16% w/v]; or two doses of sucrose [3.5, 10.5% w/v]). Wheel running and fluid consumption were measured daily, with each dose (including plain water) lasting two days and two "washout" days between solutions. In a separate set of mice, the experiment was repeated without wheel access. The artificial sweeteners had no statistical effect on wheel running. However, based on proportional responses, both doses of sucrose significantly elevated wheel running in C but not HR mice. In contrast, the high dose of sucrose suppressed home-cage activity for both linetypes. Both sucrose and the artificial blends generally increased fluid consumption in a dose-dependent manner. When they had access to wheels, HR had a significantly smaller increase in consumption of artificial sweetener blends when compared with C mice, but not when housed without wheels. Overall, these results provide further evidence that the reward system of HR mice has evolved, and specifically suggest that HR mice have a reduced incentive salience for some artificial sweetener blends, likely attributable to the stronger competing reward of wheel running that has evolved in these lines.

## 1. Introduction

Generally speaking, the mammalian brain has evolved in such a way that it reinforces behaviors that benefit the individual, while directing the individual to avoid detrimental stimuli. The reward pathway in the brain is responsible for integrating a myriad of sensory information and for conferring an incentive salience onto some behaviors, so that those behaviors are reinforced (Berridge and Robinson, 1998; Schultz, 1998). We use "incentive salience" in the manner proposed by Berridge and Robinson (1998) (p. 313), who describe the attribution of incentive salience to an otherwise neutral stimulus as transforming "the neural representation of a stimulus into an object of attraction that animals will work to acquire." This perspective distinguishes between motivational ("wanting") and hedonic ("liking") aspects of reward. In the present study, we use the term incentive salience to underscore the motivational or "wanting" aspect of reward, because this is the component that compels the organism to seek out the reward (Berridge and Valenstein, 1991; Robinson and Berridge, 1993).

The mesocorticolimbic pathway connects the ventral tegmental area, the ventral striatum, and the prefrontal cortex (Waxman, 2010). This dopaminergic pathway, which comprises part of the reward pathway of the mammalian brain, is known to convey an incentive salience to such diverse activities as listening to music (Menon and Levitin, 2005), social-play behavior (Vanderschuren et al., 1997), gambling (Dodd et al., 2005), sexual behavior (López and Ettenberg, 2002), drug abuse (Wise and Hoffman, 1992), and pair-bonding (Aragona et al., 2003). It is also a primary component in the search for and acquisition of palatable substances (Rada et al., 2005). Moreover, administering dopamine receptor antagonists attenuates the incentive salience of a palatable reward (Baker et al., 2003). Although much is known about the reward system in mammals, many questions remain, especially concerning the comparison of behavioral rewards (e.g., is a food reward equivalent to the reward for sex?). To what extent can one rewarding behavior substitute for another? How does the strength of a

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reward influence its ability to substitute for another reward?

In humans and rodents, some rewarding substances have been shown to partially substitute for other rewarding substances or behaviors (Marcus et al., 1999; Silva and Heyman, 2001; Ussher et al., 2001, 2004; Schulze et al., 2002; Belke et al., 2006; Ozburn et al., 2008; Belke and Pierce, 2009; Van Rensburg et al., 2009). For example, in rats, consumption of a saccharin solution lowers cocaine consumption (Schulze et al., 2002) and chronic self-administered morphine consumption decreases wheel running and wheel running-reinforced lever pressing (Silva and Heyman, 2001). Moreover, there is evidence that reward substitution is not reciprocal between reinforcers. Belke et al. (2006) found that in male Wistar rats, sucrose can partially substitute for wheel running but not vice versa. Likewise, in ethanol-preferring C57BL/6J mice, access to wheel running did not change ethanol consumption, but wheel running increased following cessation of ethanol consumption (Ozburn et al., 2008). The fact that rewards are ascribed a relative value and, moreover, that one reward can partially substitute for another is exploitable. For example, in humans, bouts of moderate exercise can help attenuate symptoms involved in both nicotine and alcohol withdrawal, including the "desire" and/or "craving" for these substances. This occurs putatively by substituting the reward gained from exercise for the desire (i.e., reward seeking) for the substance of abuse (Ussher et al., 2001, 2004; Daniel et al., 2004; Taylor et al., 2007).

For more than 80 generations, we have conducted an artificial selection experiment for high voluntary wheel running with four replicate lines of laboratory house mice (Swallow et al., 1998, 2009; Wallace and Garland, 2016). The response to selection on voluntary running has been striking, with an average 2.5–3.0-fold increase in wheel running in the high runner (HR) lines as compared with four non-selected control (C) lines. Previous work has shown that HR mice have increased their daily revolutions primarily by increasing running speed instead of overall time spent running, especially for females (Swallow et al., 1998; Koteja et al., 1999). As might be expected, the HR mice show elevated endurance (Meek et al., 2009) and aerobic capacity (Rezende et al., 2006; Kolb et al., 2010) when tested with forced exercise on a motorized treadmill. The HR mice also exhibit reduced body mass (Swallow et al., 1999, 2001; Meek et al., 2009), lower body fat (Swallow et al., 2001), and changes in a variety of subordinate traits – many presumed to enhance sustained, aerobic endurance capacity – including a larger heart ventricle mass (Rezende et al., 2006; Kolb et al., 2010), elevated GLUT-4 transporter plasticity in skeletal muscle (Gomes et al., 2009), a larger femoral head (Garland and Freeman, 2005; Kelly et al., 2006), and more symmetrical hind limb bones (Garland and Freeman, 2005).

In addition to alterations in locomotor capacity, alterations in the dopaminergic (Rhodes and Garland, 2003) and endocannabinoid (Keeney et al., 2008; Thompson et al., 2017) systems have been observed in HR mice. Moreover, in an experiment in which wheel access was provided for six days and then suddenly denied, HR mice had a differential activation of brain regions known to be involved in reward, motivation, and anxiety (Rhodes et al., 2003). However, despite these indications that the reward system is altered in HR mice, a behavioral test to assay putative alterations in incentive salience has remained elusive (Belke and Garland, 2007).

Voluntary wheel running in rodents is self-rewarding (Premack et al., 1964; Timberlake and Wozny, 1979; Belke and Heyman, 1994; Belke, 1996; Sherwin and Nicol, 1996; Sherwin, 1998; Belke and Garland, 2007). The incentive salience of this activity has been quantified, in both the HR mice and other rodents, via various methodologies including increasing the cost of the reward (Sherwin and Nicol, 1996); classical operant conditioning (Belke and Heyman, 1994; Belke, 1996; Belke and Garland, 2007); and operant conditioning with consummatory reinforcers (Premack et al., 1964; Timberlake and Wozny, 1979). If wheel running has a higher incentive salience in HR mice, then the response to a competing reward should be blunted.

Palatable taste rewards have a high incentive salience in mice and directly elevate dopamine levels in the reward pathway (Hernandez and Hoebel, 1988). Therefore, a sweet solution (Flaherty and Mitchell, 1999; Spangler et al., 2004; Liu and Grigson, 2005) should act as a competing reward with wheel running, and should partially substitute for the reward gained from that behavior (Belke and Hancock, 2003). Accordingly, in the present study, we administered sucrose as well as artificial sweeteners to separate the rewarding effects of a sweet-tasting solution from the energetic effects provided by a metabolizable sugar. The metabolic effects of artificial sweeteners are negligible because these compounds maximize sweetness at very low concentrations and caloric doses (Table 1). Although consumption of artificial sweeteners varies among strains and among individuals, in general mice readily consume artificial sweeteners at low concentrations and exhibit a parabolic dose-dependent response. In this dose response, consumption initially increases up to a plateau, and then decreases at higher concentrations at which the bitterness of the compound overwhelms additional increases in sweetness (Bachmanov et al., 2001). Therefore, even at very low concentrations (< 1% w/v), such as those used in the present study (see Section 2.3), artificial sweeteners have a high relative sweetness (Table 1). Thus, using artificial sweeteners maximizes the incentive salience of the sweet taste while minimizing the post-digestive metabolic effects. By juxtaposing two rewarding stimuli – voluntary exercise and a highly palatable solution – we attempted to evaluate alterations in the incentive salience of both exercise and a sweet taste in HR and C mice.

**Table 1**
Composition of the artificial sweeteners used in this study.

| Sweetener | Primary sweetener | Secondary compounds | Secondary compound purpose | Relative sweetness[a] |
|---|---|---|---|---|
| Saccharin | sodium saccharin | None | | 300 |
| Sweet 'n Low | sodium saccharin | Dextrose[b] | bulking agent | 300 |
| | | cream of tartar | anti-caking agent | |
| | | calcium silicate | anti-caking agent | |
| Equal | aspartame | Dextrose | bulking agent | 200 |
| | | Maltodextrin | bulking agent | |
| Splenda | sucralose | Dextrose | bulking agent | 650 |
| | | Maltodextrin | bulking agent | |

[a] Relative sweetness relationships were adapted from Kirk-Othmer Encyclopedia of Chemical Technology and Ellis (1995), and are scaled to sucrose (sucrose = 1).

[b] Although dextrose and maltodextrin are added to the artificial sweetener blends primarily for bulking purposes, they are also sugars themselves – dextrose is a monosaccharide and maltodextrin is a polysaccharide of glucose.

## 2. Materials and procedures

### 2.1. Experimental animals

Mice for this study were initially derived from a base population of 224 outbred Hsd:ICR mice (*Mus domesticus*) purchased from Harlan Sprague Dawley (Indianapolis, IN). After random mating for two generations, mice were randomly allotted into 8 lines, four designated as high runner (HR) and four as non-selected control (C) lines. The HR lines have been bred for high wheel revolutions on days 5 and 6 of a 6-day period of wheel access, while the C lines have been bred without regard to the amount of running during the test (Swallow et al., 1998). Each generation, mice are wheel-tested at approximately 6–8 weeks of age, and within-family selection is used in choosing breeders. During wheel testing, mice are individually housed with access to Wahman-type running wheels (1.12 m circumference; Lafayette Instruments,

Lafayette, Indiana, USA). A minimum of 10 mating pairs from each line produces litters every generation, and pups are weaned at 21 days of age. Females were used for the present study because they run more total revolutions/day and at higher speeds than males (Swallow et al., 1998, 1999; Koteja et al., 1999; Rhodes et al., 2000; Girard et al., 2001; Rezende et al., 2006), and for consistency with previous behavioral studies aimed at elucidating reward alterations (Belke, 1996). For the saccharin, Splenda®, and sucrose trials with wheels, 72 mice (N = 9/line) from generation 42 were tested. For the Sweet 'N Low® and Equal® trials with wheels, 75 mice (approximately 9 per line) from generation 53 were tested. For all measurements without wheels, 100 mice (approximately 12 per line) from generation 75 were tested. Different generations were used for logistical reasons.

### 2.2. Experimental design

Five compounds were tested in this study: saccharin, Sweet 'N Low®, Equal®, Splenda®, and sucrose. Each was combined with tap water at two concentrations and administered to the mice daily during the trial period.

For generations 42 and 53, a wheel-access acclimation period of two weeks was given to all subjects, beginning at 9–11 weeks of age. For generation 75, mice were allowed to acclimate to single-housing for two weeks before the solutions were administered. Wheel running was recorded for 23 h/day throughout this period and for the remainder of the study, as previously described (Swallow et al., 1998). For generation 75, home-cage activity was recorded using infrared sensors for 23 h/day (Acosta et al., 2015; Thompson et al., 2017) for the portion of the study when solutions were administered. During the acclimation period, all mice had ad libitum access to tap water. Water bottles were weighed when changing solutions. Additionally, bottles were placed in empty cages to serve as evaporative controls. These bottles were weighed when solutions were changed, and changes in mass due to evaporation were recorded, averaged, and subtracted from the fluid-consumption data. Water or fluid consumption was then calculated as the difference in daily water bottle mass. Food (Harlan Teklad Rodent Diet [W] 8604, Madison, WI, USA) was provided ad libitum throughout the study.

Mice from generation 42 were given saccharin, Splenda®, and sucrose, whereas those from generation 53 were given Sweet 'N Low® and Equal®. All mice in generation 75, which were tested without wheel access, received all compounds. Within every trial (1 trial = 1 sweet solution), each mouse was randomly assigned to one of six treatment batches. The six treatment batches each included a 2-day trial at each concentration level (e.g., for saccharin: 0.1% and 0.2% w/v) as well as a 2-day tap water dose (sham), so that the total trial length for each solution was 6 days. The six treatment batches included every combination of low dose, high dose, and water "sham" dose. Between solutions, mice were given 2–4 "washout" days during which they received only tap water (Ramirez and Fuller, 1976). Thus, as an example for an individual mouse in the generation 43 cohort, the testing sequence during the saccharin trial might have been as follows: 2 days – low dose saccharin, 2 days – tap water, 2 days – high dose saccharin.

### 2.3. Palatable solutions

Sodium saccharin (for generation 42: Alfa Aesar; Ward Hill, MA, for generation 75: Acros Organics; NJ) solutions of 0.1% (low dose) and 0.2% (high dose) were used because of the known palatability of these concentrations across many strains of mice (Pelz et al., 1973; Fuller, 1974; Blizard et al., 1999). The artificial sweetener blends Sweet 'N Low® (Cumberland Packing Corp., Brooklyn, NY), Equal® (Merisant, Chicago, IL), and Splenda® (McNeil Nutritionals, Fort Washington, PA) were purchased over-the-counter. Sweet 'N Low® solutions were constituted to a 0.1% (low dose) and 0.2% (high dose) saccharin concentration, and thus, at parity with the sodium saccharin solutions. Equal® concentrations were 0.04% (low dose) and 0.08% (high dose). Splenda® concentrations were 0.08% (low dose) and 0.16% (high dose). These concentrations were based on the relative per gram concentrations of aspartame and sucralose in each sweetener blend. Artificial sweetener concentrations were chosen based on maximum concentration preference in studies of inbred strains (Pelz et al., 1973; Ramirez and Fuller, 1976; Blizard et al., 1999; Bachmanov et al., 2001). Sucrose (for generation 42: C&H Sugar Company, Crockett, CA, for generation 75: Fisher Scientific) concentrations were 3.5% (low dose) and 10.5% (high dose), based on a number of studies that have shown that sucrose consumption is maximized at doses between 2%–12% w/v (Bachmanov et al., 2001; Spangler et al., 2004; Lewis et al., 2005; Belke et al., 2006).

Commercial artificial sweetener blends (as opposed to pure aspartame, sucralose, etc.) were included in this study based on results from Dr. Craig Davis (personal communication) indicating that some laboratory mice have a strong preference for Sweet 'N Low®. Artificial sweetener blends also maximize sweetness while avoiding the bitter tastes that tend to occur at high concentrations with pure artificial sweeteners (see Dess et al., 2008 for a discussion of rats preferring Splenda to its main sweetener sucralose). In addition, using commercially available blends maximizes the relevance of our results to humans.

### 2.4. Statistical analyses

The analyses were performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA) or SAS 9.4 statistical software package (SAS Institute Inc., Cary, NC, USA) using the PROC MIXED procedure. Wheel running and fluid consumption were analyzed as 2-day averages (corresponding to the 2-day concentration trials). Two-way, repeated-measures, nested ANCOVAs, in which linetype (HR vs. C) and solution concentration were the main effects, were used to analyze the dependent variables of wheel running and fluid consumption. The statistical design of the ANCOVA included line as a random effect nested within linetype (fixed effect), with degrees of freedom always 1 and 6 for testing the effects of linetype, dose, and the linetype*dose interaction. A compound symmetry covariance structure was used in all the repeated-measures analyses. In the wheel-running analyses, wheel freeness and age were used as covariates. In the fluid-consumption analyses, body mass and age were used as covariates. Fluid-consumption data for all sweeteners were log transformed to improve normality of residuals. Wheel-running data were not transformed.

For all of the statistical analyses, P values were for two-tailed tests and were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Biometric data

Mice from generation 42 were 61–76 days old (mean = 73, S.D. = 3), mice from generation 53 were 71–75 days old (mean = 73, S.D. = 1), and mice from generation 75 were 58–72 days of age (mean = 69, S.D. = 2) at the start of each experiment. HR mice were smaller than C mice, as has been reported previously (Swallow et al., 1999, 2001; Meek et al., 2009). Average body mass (simple means ± S.E.) for HR and C mice, respectively, was 25.1 ± 0.4 g and 27.1 ± 0.4 g in generation 42, and 25.7 ± 0.4 g and 28.4 ± 0.5 g in generation 53, and 25.2 ± 0.3 g and 28.6 ± 0.3 g in generation 75. This difference in size between HR and C lines emphasizes the importance of using body mass as a covariate in analyses of fluid consumption.

### 3.2. Wheel running

The ratio of total wheel revolutions (HR/C) on the last 2 days of acclimation (preceding the treatment phase) was 2.8 (12,361/4400

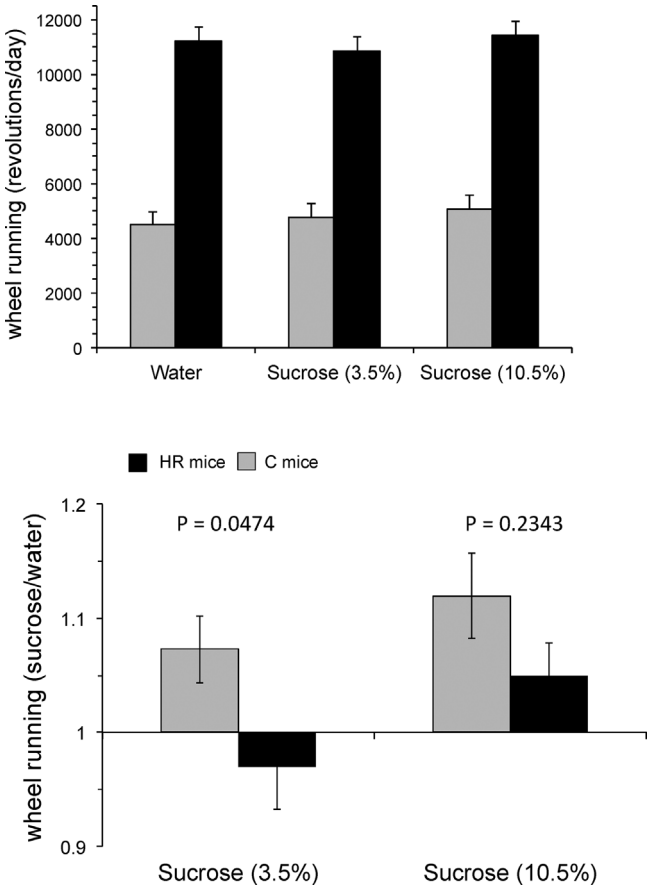


**Fig. 1.** Wheel running during sucrose trials, absolute and proportional responses.
A) Wheel revolutions during sucrose trials (least squares means ± S.E. derived from a repeated-measures, two-way ANCOVA with linetype and sucrose dose as the main effects). Gray bars are C mice and black bars are HR mice. Effects of linetype ($P < 0.0001$) and sucrose dose ($P = 0.0386$) were statistically significant, but their interaction was not ($P = 0.1598$; see Table 2). Sucrose was the only substance that had a statistically significant effect on wheel revolutions (Table 2). Averaging across HR and C mice, least squares means ( ± S.E.) for wheel revolutions were 7863 ( ± 353), 7819 ( ± 353), and 8268 ( ± 353) for water, low dose, and high dose sucrose. Thus, high-dose sucrose increased wheel revolutions by 5.4% relative to the average values for water and low-dose sucrose. B) Proportional response in voluntary wheel running (revolutions/day) during sucrose trials. Values are least squares means ( ± S.E.) from separate one-way ANCOVAs of running with sucrose/with water, with linetype as the main effect and age and wheel freeness as covariates (p-values are for the linetype effect). See Results Section 3.2 for statistical details.

revolutions) in generation 42 and 2.3 (9777/4268 revolutions) in generation 53 (simple means). The average ratio across both generations was 2.6. This ratio remained similar when mice were administered sweet solutions (see Fig. 1A for an example).

When given the various solutions, solution concentration for the artificial sweeteners had no detectable effect on wheel revolutions, with no statistical interactions between linetype and solution concentration (Table 2, repeated-measures, two-way nested ANCOVAs). However, sucrose increased total wheel revolutions (Table 2, dose $P = 0.0386$). Separate one-way ANCOVAs of the revolutions run at individual doses (Fig. 1B) indicated that the proportional response of C mice was significantly higher than for HR mice at the 3.5% sucrose dose (linetype $P = 0.0474$), but not at the 10.5% sucrose dose (linetype $P = 0.2343$). Moreover, based on comparisons of the estimated least-squares means with zero, the increased running by C mice given sucrose was statistically significant for both doses (3.5%: +7.3%, $P = 0.0484$; 10.5%: +12.0%, $P = 0.0187$), whereas it was not for HR mice (3.5%: −3.0%, $P = 0.3361$; 10.5%: +4.9%, $P = 0.2345$).

The number of 1-min intervals with any wheel revolutions did not differ between HR and C mice, nor did they vary with regard to concentration for the artificial sweetener treatments (repeated-measures, two-way nested ANCOVAs: results not shown). Sucrose, however, increased the number of 1-min intervals run in a dose-dependent manner ($P = 0.0058$), and there was a marginal interaction between linetype and dose ($P = 0.0580$), with the increase being somewhat greater in the C mice. Thus, sucrose increased wheel revolutions in the C mice, and this was primarily due to an increase in the time spent running. Finally, the average and maximum running speeds (RPMs) were always higher in HR mice (repeated-measures, two-way nested ANCOVAs: results not shown), but were not affected by any of the sweeteners.

### 3.3. Consumption of sweeteners with wheel access

The two-way, repeated-measures, nested ANCOVAs indicated that saccharin significantly increased fluid consumption in both HR and C mice, at both doses (Table 2, Fig. 3A). Sweet 'N Low, Equal, and Splenda also increased fluid consumption, but with significant linetype*dose interactions (Table 2), such that the increases with dose were greater for C mice than for HR mice (Fig. 3, left panels). Sucrose also increased fluid consumption with wheel access for both HR and C mice (Table 2, Fig. 3I), but the linetype*dose interaction caused a somewhat different pattern than for the artificial sweeteners, with HR mice tending to drink more than C for water and high dose, but not for low dose. Analyses of proportional responses yielded similar results (Supplementary Fig. 1).

### 3.4. Home-cage activity

When housed without wheels, HR mice always had higher home-cage activity (total activity per day) than C mice (Table 2, all linetype $P < 0.006$), regardless of which solution they were consuming. The higher activity of HR mice was attributable to both the duration (for linetype, saccharin $P = 0.0066$, Sweet 'N Low $P = 0.0050$, Equal $P = 0.0205$, Splenda $P = 0.0086$, sucrose $P = 0.0045$) and the average intensity of activity when mice were active (for linetype, with all values log-transformed to improve normality of residuals, saccharin $P = 0.0100$, Sweet 'N Low $P = 0.0024$, Equal $P = 0.0052$, Splenda $P = 0.0051$, sucrose $P = 0.0052$).

Dose of the artificial sweeteners did not have a statistically significant effect on home-cage activity, and we observed no linetype*dose interactions (Table 2). However, sucrose dose did affect activity ($P = 0.0203$), primarily due to a suppression of activity by the higher dose of sucrose (Fig. 2B). Sucrose dose significantly affected the average intensity of activity ($P = 0.0020$, lowest at the higher dose), but not the duration of activity ($P = 0.1544$), with no significant linetype*dose interaction ($P = 0.49$ for intensity, $P = 0.75$ for duration). Separate analyses of the proportional changes in home-cage activity at individual doses (Fig. 2B) indicated that the proportional response did not differ between HR and C mice for either sucrose dose (linetype $P = 0.8156$ and $P = 0.8562$, respectively), and neither HR nor C mice differed significantly from unity for either dose.

### 3.5. Consumption of sweeteners without wheel access

Without wheels, dose significantly affected fluid consumption for all solutions (all $P < 0.0001$: Table 2), and as shown in Fig. 3, mice always drank more of the sweet-tasting solutions than they did water. However, without access to wheels (Fig. 3 right panels), none of the linetype*dose interactions were significant (all $P > 0.37$: Table 2), which contrasts sharply with the results when mice had wheel access (Fig. 3, left panels). Analyses of proportional responses yielded similar results (Supplementary Fig. 1).

**Table 2**
Significance levels for voluntary wheel-running behavior, home-cage activity (in the absence of wheels), and fluid consumption (log-transformed) based on repeated-measures, two-way nested ANCOVAs.

| Treatment | Wheel Running (revolutions/day)[a] | | | Fluid Consumption With Wheel Access[b] | | |
|---|---|---|---|---|---|---|
| | $P_{Linetype}$ | $P_{Dose}$ | $P_{Linetype \times Dose}$ | $P_{Linetype}$ | $P_{Dose}$ | $P_{Linetype \times Dose}$ |
| Saccharin | < **0.0001** | 0.8552 | 0.3755 | 0.2155 | < **0.0001** | 0.7692 |
| Sweet 'N Low | **0.0031** | 0.6728 | 0.3627 | 0.0903 | < **0.0001** | **0.0172** |
| Equal | **0.0040** | 0.6869 | 0.3208 | **0.0324**[c] | < **0.0001** | **0.0116** |
| Splenda | < **0.0001** | 0.9909 | 0.9525 | 0.2518 | < **0.0001** | **0.0076** |
| Sucrose | < **0.0001** | **0.0386** | 0.1598 | 0.2792 | < **0.0001** | **0.0226** |

| Treatment | Home-cage Activity (total/day)[d] | | | Fluid Consumption Without Wheel Access[b] | | |
|---|---|---|---|---|---|---|
| | $P_{Linetype}$ | $P_{Dose}$ | $P_{Linetype \times Dose}$ | $P_{Linetype}$ | $P_{Dose}$ | $P_{Linetype \times Dose}$ |
| Saccharin | **0.0010** | 0.1691 | 0.4708 | 0.5075 | < **0.0001** | 0.3688 |
| Sweet 'N Low | **0.0012** | 0.8833 | 0.6206 | 0.7123 | < **0.0001** | 0.7218 |
| Equal | **0.0054** | 0.6899 | 0.5970 | 0.5959 | < **0.0001** | 0.7405 |
| Splenda | **0.0036** | 0.7193 | 0.8981 | 0.5491 | < **0.0001** | 0.8699 |
| Sucrose | **0.0012** | **0.0203** | 0.9207 | 0.5544 | < **0.0001** | 0.6056 |

*P* values were considered significant at $P < 0.05$ (in bold).
[a] For wheel running, age and wheel freeness were used as covariates, but neither was ever statistically significant (results not shown).
[b] In the fluid consumption analyses, age and body mass were used as covariates (results not shown).
[c] HR mice drank significantly less of both doses of Equal, but not of water.
[d] For home-cage activity, age and sensor sensitivity were used as covariates, but neither was ever statistically significant (results not shown).

## 4. Discussion

The main purpose of this study was to explore reward substitution in the context of voluntary exercise, by use of a novel mouse model. Natural and non-nutritive sweeteners were offered as competing rewards for wheel running, and we studied females from four replicate High Runner lines, selectively bred for voluntary wheel running, as well as four non-selected Control lines. Our first general result was that none of the artificial sweeteners (Table 1) had a statistically significant effect on wheel running or home-cage activity, the latter measured for mice that did not have wheel access, in either HR or C mice. However, sucrose increased wheel running in C mice without affecting the running of HR mice. In contrast, sucrose suppressed home-cage activity for both HR and C mice housed without wheels. Our second general result was that, as compared with C mice, HR mice had a significantly smaller increase in consumption for artificial sweetener blends when they had access to wheels, but not when housed without wheels. These results suggest that HR mice have a reduced incentive salience for some artificial sweetener blends, likely attributable to the stronger competing reward of wheel running that has evolved in these lines.

For C mice, sucrose increased the amount of wheel running by increasing the time spent running, rather than running speed, based on analyses of proportional responses (Fig. 1). Both C and HR mice run for many hours each night (Garland et al., 2011a), and sucrose in drinking water may serve as a fuel for that exercise. A previous study of these mice found that the sports drink Red Bull increased the amount of wheel running in both HR and C mice, and this was also a result of an increase in time spent running (Claghorn et al., 2017). When the active ingredients of Red Bull were tested separately, caffeine increased the amount of wheel running, but not to the same degree as Red Bull itself, suggesting that the sucrose in the drink also plays a role in the increased exercise behavior.

We believe it is unlikely that the increased wheel running in the C mice (but not in HR) is simply due to the HR mice being closer to a "ceiling" – as previous studies on these mice have reported higher levels of wheel running than what is reported here (e.g., see Careau et al., 2013; Kelly et al., 2014; Thompson et al., 2017).

Alternatively, the increased wheel running observed in C mice with sucrose, but not with artificial sweeteners, may arise from the differential sensory processing of natural sugars rather than their metabolic effects. A recent study by Chambers et al. (2009) in trained human cyclists found that oral rinses of a glucose solution increased maximal oxygen consumption ($VO_2$max), whereas solutions containing saccharin did not, and that those increases were associated with activation of reward-related brain regions such as the cingulate cortex and striatum. The striatum has been specifically tied to wheel running in mice (Rhodes et al., 2003; Waters et al., 2013; Saul et al., 2017). The authors hypothesized that this differential response suggested "a class of so far unidentified oral receptors that respond to carbohydrate independently of those for sweetness." Whatever the mechanism underlying the effect of sucrose on physical activity in C mice, these effects were not observed in HR mice, thus providing further evidence that they have evolved in terms of motivation and/or ability for voluntary exercise (Garland et al., 2016; Wallace and Garland, 2016).

When housed without wheels, sucrose significantly affected home-cage activity for both HR and C mice, primarily due to a suppression of activity by the high dose (Fig. 2). The high dose decreased the average intensity of activity, without affecting the number of minutes per day the mice were active. Recent studies have shown that fructose can suppress home-cage activity in rodents (Rendeiro et al., 2015) and decrease energy expenditure in humans (Cox et al., 2012). The mechanism underlying such effects is not yet known, but may involve how and where fructose is metabolized (Samuel, 2011). It may seem surprising that sucrose seemed to have an opposite effect on wheel running versus home-cage activity. Although both are activity measures, they assess different aspects of locomotor behavior. For the mice in this study, wheel running is a form of voluntary exercise, while home-cage activity better represents spontaneous physical activity or non-exercise activity thermogenesis (see Garland et al., 2011b). Thus, wheel running involves exercise physiology and limitations related to that, whereas home-cage activity does not. The factors limiting voluntary exercise in these mice (e.g., see Claghorn et al., 2016) and in other animals in various situations are not well understood. Mice from the HR lines sometimes run voluntarily on wheels at intensities that may tax their maximal aerobic capacity, whereas C mice do not (Girard et al., 2001; Rezende et al., 2009, and references therein). Thus, if sucrose is acting as a substrate for exercise metabolism, then it might allow increased running for C mice, but not for HR mice.

With the exception of saccharin (see below), all mice drank more of the sweet solutions in a dose-dependent manner, both with and without

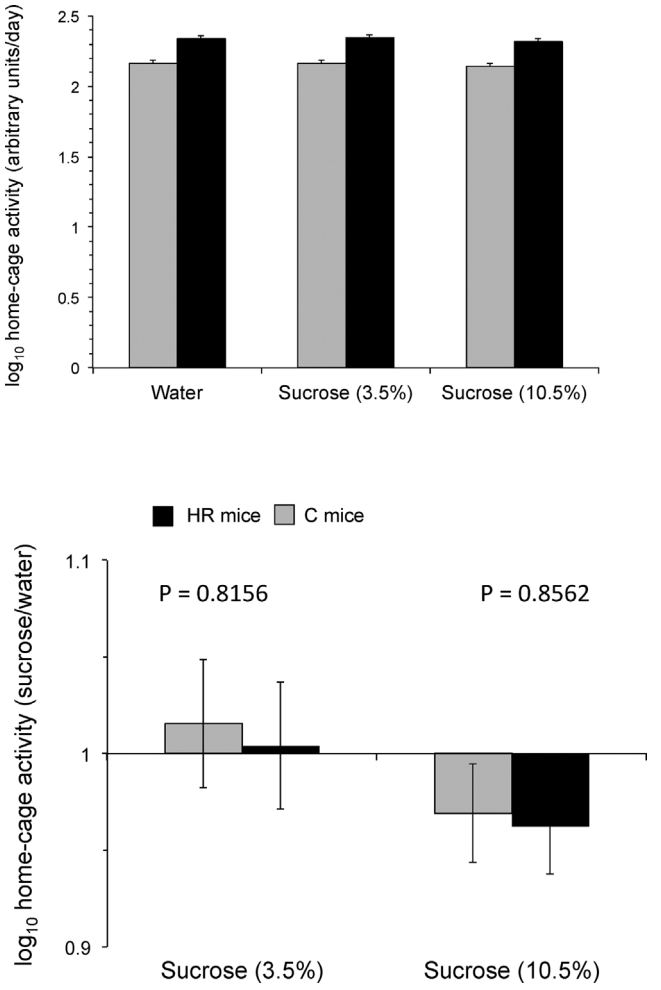


**Fig. 2.** Home-cage activity during sucrose trials, absolute and proportional responses. A) Home-cage activity during sucrose trials (least squares means ± S.E. derived from a repeated-measures, two-way ANCOVA with linetype and sucrose dose as the main effects). Gray bars are C mice and black bars are HR mice. Effects of linetype ($P = 0.0012$) and sucrose dose ($P = 0.0203$) were statistically significant, but their interaction was not ($P = 0.9207$; see Table 2). B) Proportional response in home-cage activity during sucrose trials when mice did not have access to wheels. Values are least squares means ( ± S.E.) from separate one-way ANCOVAs of activity with sucrose/with water, with linetype as the main effect and age and sensor sensitivity as covariates (p-values are for the linetype effect). See Results Section 3.4 for statistical details.

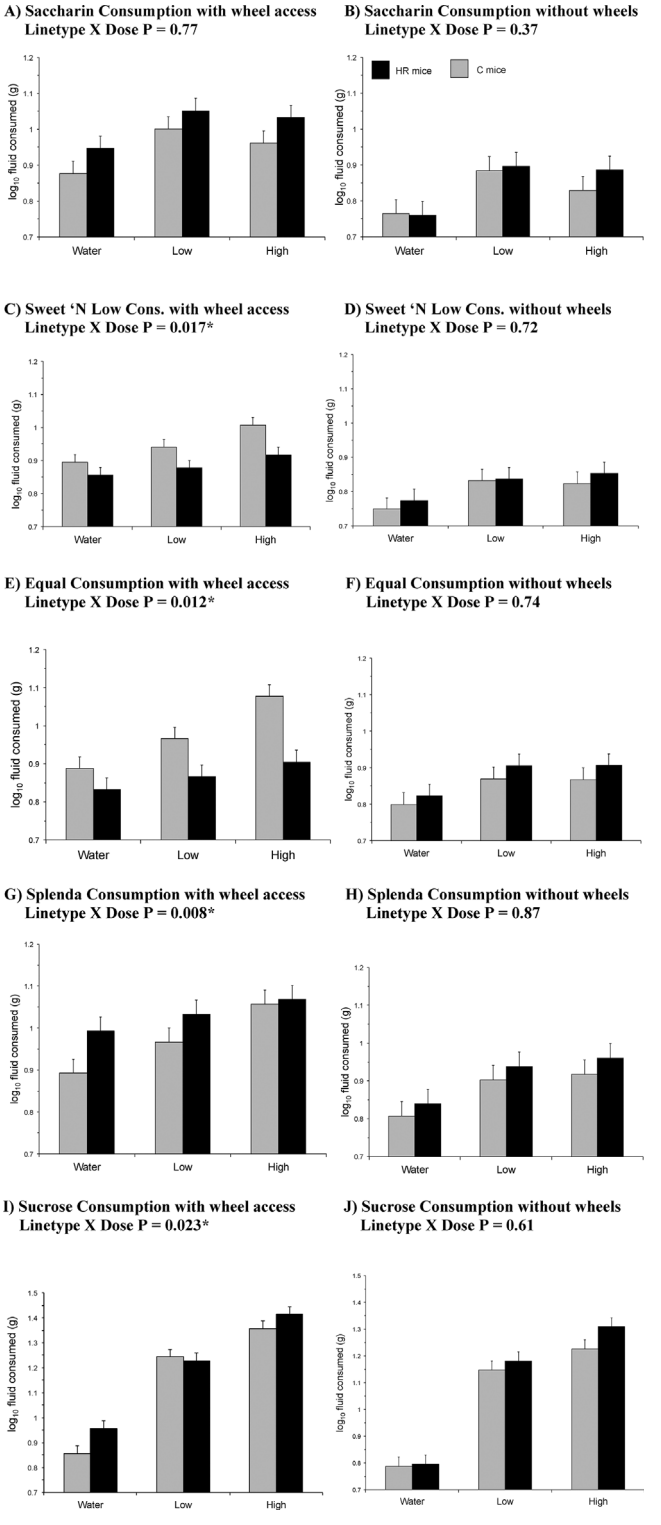


**Fig. 3.** Consumption of sweet-tasting solutions, with and without access to wheels. Consumption of sweet-tasting solutions during access to wheels (A, C, E, G, I) or without access to wheels (B, D, F, H, J). Values are least-squares means ( ± standard errors) of log-transformed data from SAS Proc Mixed (Table 2). Gray bars are C mice and black bars are HR mice. For four of the sweet solutions, when mice had wheel access the linetype*dose interaction was statistically significant (Table 2), such that the increases in consumption were mainly in C mice at the higher doses.

wheel access (Fig. 3). However, as compared with C lines, mice from HR lines had a significantly smaller increase in consumption for artificial sweetener blends when they had access to wheels, but not when housed without wheels. These results indicate that the HR lines have evolved with respect to the incentive salience of a reward (some sweet-tasting solutions) when an important competing reward (wheel running) is present. Sweet taste is the primary means by which artificial sweeteners affect the body (i.e., the caloric value is negligible), so a relative reduction in consumption of a sweet-tasting solution by HR mice is most likely the result of a lower incentive salience for the sweet-taste reward.

The effect we saw when HR mice were given wheels was a smaller increase in sweetener fluid consumption, rather than a decrease in fluid consumption. This result may be better explained by the phenomenon of cross-sensitization rather than a substitution of one reward for another (Avena and Hoebel, 2003). Because the mice had free access to food during all experiments, it is possible that HR mice may have adjusted the amount of food eaten, thereby preventing us from seeing a clean substitution between wheel running behavior and fluid consumption. Nevertheless, it is clear that reward-seeking behavior is altered in HR mice as compared with C when both linetypes are given access to wheels.

As mentioned above, and seen in Fig. 3(A,B), saccharin is the only solution that did not show a dose-dependent increase in consumption.

Saccharin is also the only non-natural compound presented as a pure substance, instead of a blend. The secondary compounds added to artificial sweetener blends can affect the taste (Dess et al., 2008), specifically by masking aversive aspects of the taste.

Previous studies of HR mice have demonstrated alterations in the dopaminergic system (Rhodes and Garland, 2003), the endocannabinoid system (Keeney et al., 2008, 2012; Thompson et al., 2017), the serotonergic system (Claghorn et al., 2016), wheel-running related differences in brain activity or gene expression, especially in regions associated with anxiety, reward, and/or anticipation (Rhodes and Garland, 2003; Garland et al., 2011b; Caetano-Anollés et al., 2016; Saul et al., 2017), and enhanced preferences for Western diet (Acosta et al., 2017). Given that the trait under selection is voluntary wheel running, it is not surprising that an elevated motivation to run may underpin some or much of the HR phenotype (Rhodes et al., 2005; Garland et al., 2011b, 2016). The reduced incentive salience for a competing reward lends support to the hypothesis that there are alterations in the reward system of HR mice, and that these alterations may have resulted in a greater incentive salience attributed to wheel running. Here we present evidence that a completely different trait (i.e., the incentive salience of a sweet taste reward) is altered in these animals. The most parsimonious explanation is that the reward system, when up-regulated for one trait, is less sensitive to a competing trait (i.e., there is a limited capacity to the reward pathway). Similarly, Belke and Garland (2007) have suggested the existence of an inherent trade-off between reward systems "tuned" for different stimulus intensities and durations (e.g., long- vs. short-duration stimuli). Moreover, the sensitivity to behavioral rewards may also be influenced by biometric factors, such as body mass (Belke and Pierce, 2009), which is lower in HR mice than in C (see Results; Swallow et al., 1998; Garland et al., 2011a).

Results of the present study emphasize the important role of motivation in voluntary exercise (see also Garland et al., 2011b). Although specific anatomical and physiological traits are necessary to sustain high levels of activity, recent studies have suggested that the brain plays an underappreciated role in regulating exercise performance and capacity (Kayser, 2003; Baden, 2005; Noakes, 2007, 2008; Rose and Parfitt, 2007; Claghorn et al., 2016). Further studies of the evolution of altered incentive salience, and its neurobiological underpinnings, are warranted.

## Conflict of interest statement

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2017.11.004.

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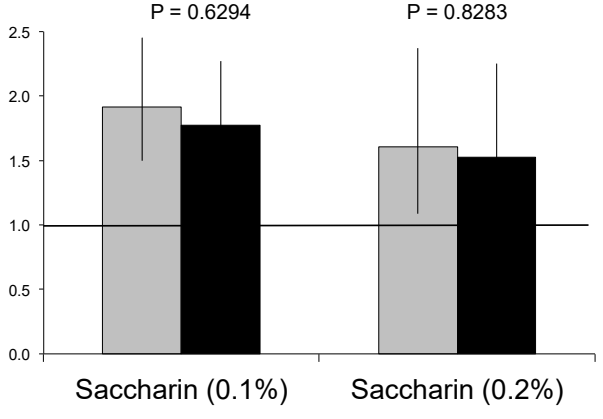
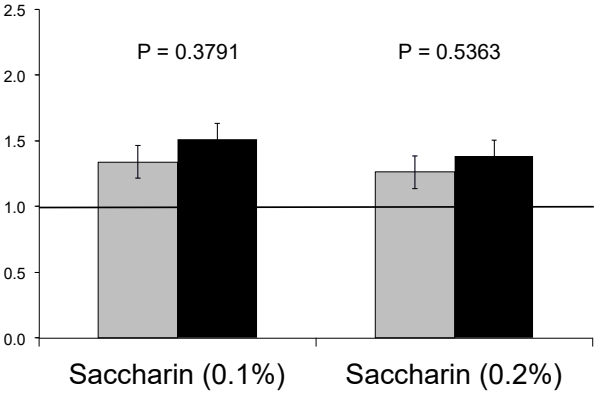
# Supplementary Materials

**Supplementary Figure 1.**
Proportional changes in fluid consumption in response to sucrose or artificial sweetener administration from nested, one-way ANCOVAs of each dose with body mass as a covariate (SAS Procedure Mixed). The proportional data presented are ratios of sweetener consumption to water consumption at each sweetener dose. P values are for the effect of linetype and are in bold if $P<0.05$. Shown for the left panels (with wheel access) are least squares means and 95% C.I. Shown for the right panels (without wheel access) are least squares means and standard errors. Light-colored bars indicate C mice and dark-colored bars indicate HR mice. A reference line has been drawn to demarcate a ratio of 1.

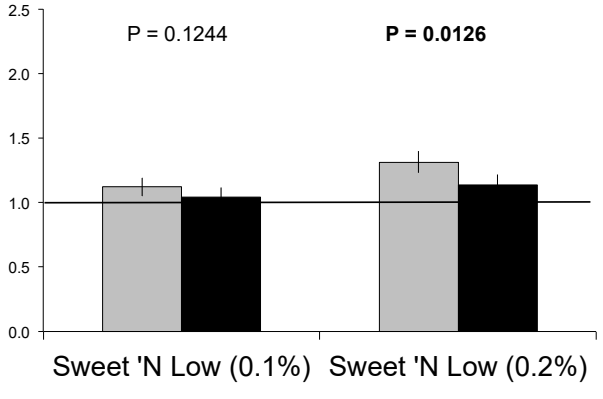
**A) Proportional Saccharin Consumption with Wheel Access**



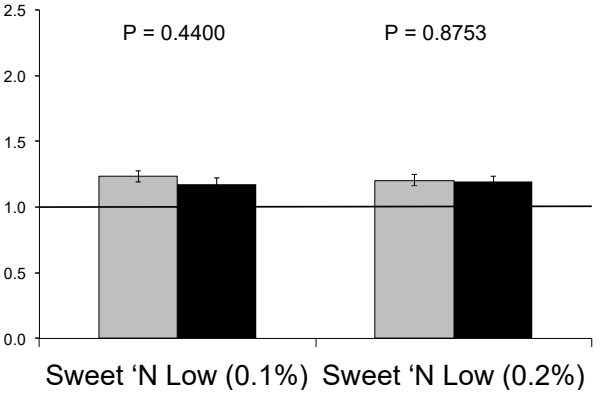

**B) Proportional Saccharin Consumption without Wheel Access**



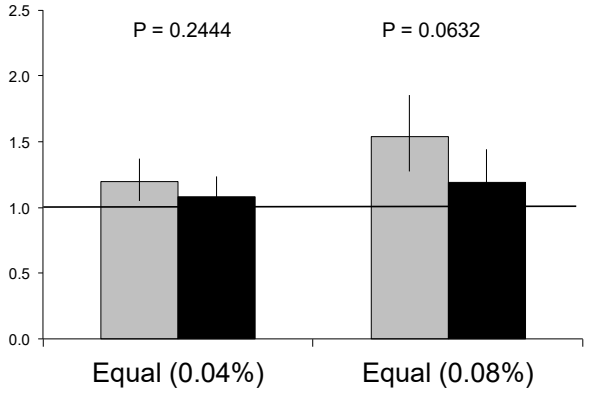

**C) Proportional Sweet 'N Low Consumption With Wheel Access**



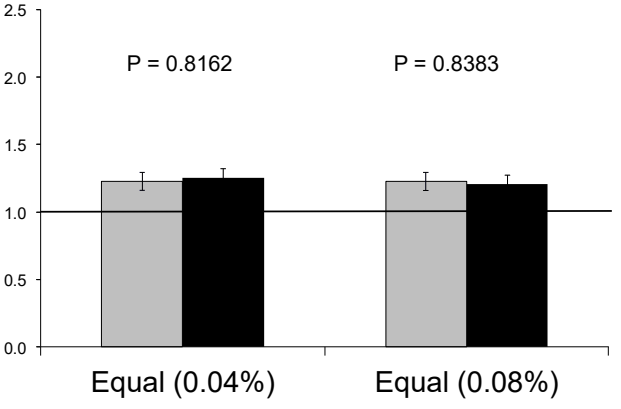

**D) Proportional Sweet 'N Low Consumption without Wheel Access**



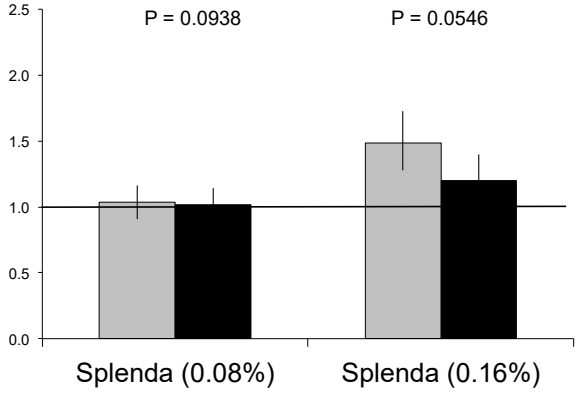
**E) Proportional Equal Consumption with Wheel Access**



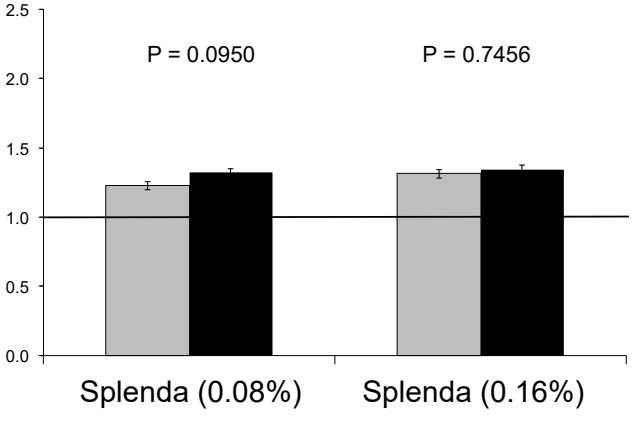

**F) Proportional Equal Consumption without Wheel Access**



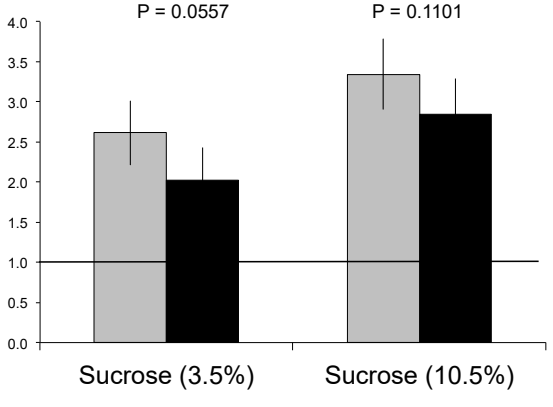

**G) Proportional Splenda Consumption with Wheel Access**




**H) Proportional Splenda Consumption without Wheel Access**




**I) Proportional Sucrose Consumption with Wheel Access**




**J) Proportional Sucrose Consumption without Wheel Access**